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A contribution to the systematics of Alpine species of the genus *Erebia* (Nymphalidae: Satyrinae)

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Summary. The genetic relationships of 21 alpine *Erebia* species are investigated by enzyme electrophoresis based on 15 enzyme loci. The results are presented as a phenogram, using coefficients of genetic similarity I (Nei, 1972) as a matrix for cluster analysis (UPGMA). High levels of genetic similarity are found for 1. *cassioides*, *nivalis* and *tyndarus*, 2. *pluto* and *gorge*, 3. *euryale* and *manto*, 4. *aethiops* and *alberganus*, 5. *melampus* and *sudetica*. Other species or groups of species cluster at rather low levels of genetic similarity (I = 0.60–0.40). The discussion includes a comparison with controversial species groups suggested by other investigators.

Zusammenfassung. Die genetischen Verwandtschaftsbeziehungen zwischen 21 alpinen Arten der Schmetterlingsgattung *Erebia* wurden durch elektrophoretische Analyse von 15 Enzymloci untersucht. Die Resultate sind in Form eines UPGMA-Phenogrammes, basierend auf einer Matrix von genetischen Ähnlichkeiten nach Nei (1972), dargestellt. Eine hohe genetische Ähnlichkeit wurde zwischen folgenden Taxa beobachtet: 1. *cassioides*, *nivalis* und *tyndarus*, 2. *pluto* und *gorge*, 3. *euryale* und *manto*, 4. *aethiops* und *alberganus*, 5. *melampus* und *sudetica*. Zwischen den restlichen Taxa oder Gruppen von Taxa lagen die Ähnlichkeitswerte deutlich tiefer (I = 0.60–0.40). In der Diskussion werden die erhaltenen Gruppierungen mit denjenigen anderer Autoren verglichen.

Résumé. Les relations génétiques entre 21 espèces alpines appartenant au genre *Erebia* ont été établies sur base d'analyses électrophorétiques de 15 locus d'enzymes. Les résultats sont présentés sous forme d'un phénogramme, obtenu par agglomération hiérarchique (UPGMA) à partir de ressemblances génétiques I (Nei, 1972) entre les taxons. Un haut degré de ressemblance génétique est observé pour les taxons suivants: 1. *cassioides*, *nivalis* et *tyndarus*, 2. *pluto* et *gorge*, 3. *euryale* et *manto*, 4. *aethiops* et *alberganus*, 5. *melampus* et *sudetica*. Pour d'autres espèces ou groupes d'espèces ce degré de ressemblance génétique est beaucoup plus faible (I = 0.60–0.40). Dans la discussion, les résultats obtenus pour les groupes d'espèces controversés sont comparés à ceux de travaux d'autres auteurs.

Key words: *Erebia*, alpine species, genetic similarity, enzyme electrophoresis, Europe.

Introduction

The genus *Erebia* Dalman, 1816 is one of the largest genera of European butterflies. Most species live in alpine habitats, many of them are endemic to the Alps and have a rather restricted range. A starting point and a fundamental base of the *Erebia* study is still a monograph of the genus by Warren (1936). More recently, the systematics received considerable attention of an extended body of students (Lorković, 1975; Roos & Arnscheid, 1979a, 1979b, 1980a, 1980b; Sonderegger, 1980; Geiger & Rezbanyai, 1982; Lukhtanov, 1987; Lattes *et al.*, 1992; Cupedo, 1996, 1997), with special emphasis on the Alpine species. Roos & Arnscheid (1979a) discussed certain speciation phenomena in this genus and, based on morphological and ecological studies, sorted out species complexes comprising "closer but not closest related species". Sonderegger (1980) arrived at controversial conclusions, working mainly on characters of genitalia and the shape of the cremaster. A different approach, the analysis of genetic relationships of the *Erebia* species, based on data from enzyme electrophoresis, was published by Geiger & Rezbanyai (1982) and Lattes *et al.* (1992). We have resumed the former study and have increased the number of species investigated and sample sizes analysed. As our investigation is continuing, however, we feel the present data are of significance.

Material and methods

The taxa studied, origin of material and sample sizes are listed in Table 1. With the exception of the *E. meolans* samples, the various conspecific samples from Grindelwald were obtained several kilometers apart, on spots separated by topographical and/or ecological barriers, and can therefore be regarded as belonging to separate populations. As the samples of *meolans* did not differ in their allelic frequencies, they were pooled for further analysis. In addition to 21 Alpine species, we have included *E. epipsodea* from Alaska. According to Warren (1936), this species does not resemble any other species of the genus and was therefore used as an outgroup. Specimens were stored in liquid nitrogen or in a deep freezer until used for electrophoresis.

The electrophoretic methods are essentially those of earlier studies in our laboratory (e.g. Scholl *et al.*, 1978; Geiger & Scholl, 1985).

Table 1. Taxa investigated, origin and sample sizes (a, b, c refer to different populations in the same area).

Taxa	Sampling sites		Sample size
E. ligea (Linnaeus, 1758)	Grindelwald BE	a	14
		b	14
E. euryale isarica Heyne, 1895	Grindelwald BE		9
E. manto ([Denis & Schiffer-müller], [1775])	Grindelwald BE	a	16
		b	12
		c	15
E. flavofasciata Heyne, 1895	Campolungo–Pass TI		14
E. epiphron aetherius (Esper, [1805])	Grindelwald BE	a	12
		b	16
E. pharte (Hübner, [1804])	Grindelwald BE	a	14
		b	14
E. melampus (Fuessly, 1775)	Schwarzwaldalp BE		13
E. sudetica Staudinger, 1861	Grindelwald BE	a	17
		b	9
		c	7
E. aethiops (Esper, [1777])	Grindelwald BE	a	13
		b	14
		c	12
E. alberganus (de Prunner, 1798)	Campolungo–Pass TI		14
E. epipsodea Butler, 1868	Tok, Alaska		7
E. pluto (de Prunner, 1798)	Grindelwald BE		14
E. gorge (Hübner, [1804])	Grindelwald BE	a	9
		b	19
E. mnestra (Hübner, [1804])	Campolungo–Pass TI		8
E. tyndarus (Esper, [1781])	Grindelwald BE	a	18
		b	17
		c	15
E. cassioides (Reiner & Hohenwarth, 1792)	Grindelwald BE	a	15
		b	15
	Schilthorn BE		17
E. nivalis Lorković & de Lesse, 1954	Grindelwald BE	a	16
		b	16
	Schilthorn BE		17
E. pronoe vergy (Ochsenheimer, 1807)	Grindelwald BE	a	19
		b	15
E. montana (de Prunner, 1798)	Grindelwald BE	a	16
		b	14
	Campolungo–Pass TI		4
E. oeme (Hübner, [1804])	Grindelwald BE		13
E. meolans stygne (Ochsen-heimer, 1807)	Grindelwald BE	a	19
		b	11
E. pandrose (Borkhausen, 1788)	Grindelwald BE	a	17
		b	15

Electrophoretic analysis of individual specimens was carried out in vertical starch gels, using 13% starch (Connaught starch hydrolysed). The enzyme assays followed standard procedures (e.g. Ayala *et al.*, 1972; Harris & Hopkinson, 1976). In some cases they were slightly modified according to Scholl *et al.* (1978). The 15 following enzyme loci were scored (EC number and number of loci are given in brackets): adenylate kinase (EC 2.7.4.3; 2 loci), glycerol-3-phosphate dehydrogenase (EC 1.1.1.8; 1), aspartate aminotransferase (EC 2.6.1.1; 2), alanine aminotransferase (EC 2.6.1.2; 1), hexokinase (2.7.1.1; 1), phosphogluconate dehydrogenase (EC 1.1.1.44; 1), isocitrate dehydrogenase (EC 1.1.1.42; 1), malate dehydrogenase (EC 1.1.1.37; 2), malic enzyme (EC 1.1.1.40; 1), phosphoglucomutase (EC 5.4.2.2; 1), glucose-6-phosphate isomerase (EC 5.3.1.9; 1) and pyruvate kinase (EC 2.7.1.40; 1).

We have calculated coefficients of genetic identity resp. distance according to Nei (1972, 1975) between all population samples. Based on these matrices, phenograms were constructed using the neighbour-joining (Saitou & Nei, 1987) and UPGMA (Sneath & Sokal, 1973) algorithms.

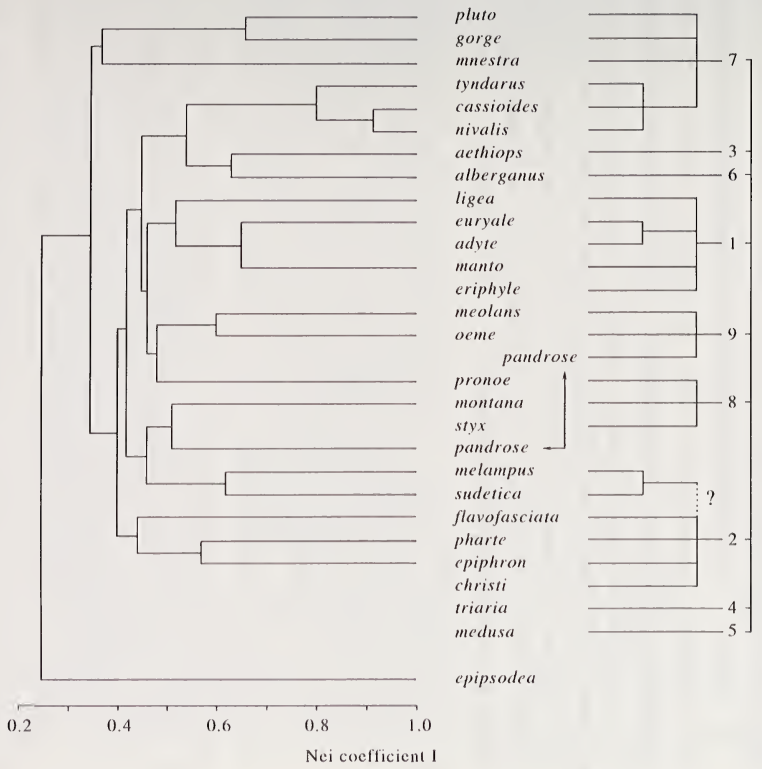
Results and Discussion

In the upper regions of the tree topology, all four phenograms analysed were nearly identical. In the lower regions however, the topologies were quite different, without showing clear tendencies. We therefore will restrict our discussion to the groupings observed in all phenograms and present only the phenogram based on the measure of Nei's genetic identity (Nei, 1972) using the UPGMA cluster algorithm (fig. 1).

Coefficients of genetic similarity which we found in comparisons of conspecific populations ranged between $I = 0.99$ (e.g. *E. meolans*, populations Grindelwald a and b; *E. tyndarus*, populations Grindelwald b and c) and $I = 0.82$ (*E. sudetica*, populations Grindelwald b and c). Conspecific populations formed distinct clusters in the phenogram. These clusters are not shown in fig. 1, since our main interest here is to compare genetic similarities beyond the species level.

The highest level of genetic similarity ($I = 0.92$) is recorded for *nivalis* and *cassioides*. This level is within the range observed in comparisons of conspecific populations, as mentioned above. *E. nivalis* and *cassioides* form a distinct cluster with *tyndarus* at the level of $I = 0.80$ (fig. 1, left). In the study by Lattes *et al.* (1992), *nivalis* is considered to be

Erebia - Dendrogram
Electrophoretic data



Erebia species groups
data of Sonderegger (1980)

Erebia species groups
data of Roos & Arnscheid (1980a)

a	b	c	d	e	f	g	h	i	k
								+	
			+						
			+						
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Fig. 1.

the most distinct of the three species *cassioides*, *tyndarus* and *nivalis*. However, the set of enzyme loci used in the two studies differs by eight loci. In addition, at two loci that were analysed in both studies, Lattes *et al.* (1992) found no activity for *nivalis* at two loci (ALAT (= GPT) and AAT-2 (= GOT-2)) for which we found clearly interpretable bands. Other species or groups of species usually cluster at levels between I = 0.60 and I = 0.40. Slightly higher genetic similarities are found for a) *pluto* and *gorge*, b) *aethiops* and *alberganus*, c) *euryale* and *manto* and d) *sudetica* and *melampus* (fig. 1). On the other hand, with respect to low levels of genetic similarity, it is of interest that the species used as an outgroup, the Alaskan *E. epipsodea*, is clearly isolated in the phenogram (fig. 1) from the European species. The taxa *pluto*, *gorge* and *mnestra* form a cluster, which is separate from all other European species investigated; however, the branching point of *mnestra* in this cluster is very low (I = 0.37).

In figure 1, we have attempted to compare the results of our electrophoretic investigation with groupings proposed by Roos & Arnscheid (1980a) and Sonderegger (1980). It is obvious that there are considerable discrepancies between the three studies and the systematics of *Erebia* need further investigation before we can sort out species groups. However, in order to describe the major discrepancies, we will here use the species group names as in Sonderegger's study (1980) for reasons of convenience:

- "*pluto* group" (7 in fig. 1). Electrophoretically, *pluto* and *gorge* show rather high similarities, but they are quite different from *mnestra* (even though these three taxa form a cluster in the phenogram) and *tyndarus* / *cassioides* / *nivalis*. In Roos & Arnscheid's studies (1979a and 1980a) the six species are placed in three groups. However, the grouping is not consistent with the electrophoresis phenogram.
- *aethiops* (3 in fig. 1) and *E. alberganus* (6 in fig. 1) show rather high electrophoretic similarities. However, in Sonderegger's study both species are separate from other species or species groups. Roos & Arnscheid have grouped *aethiops* with members of the "*ligea* group" (a in fig. 1) and *alberganus* with *oeme* and *medusa* (h in fig. 1).
- "*ligea* group" (1 in fig. 1). The taxa *adyte* and *eriphyle* were not included in the electrophoretic survey. *E. ligea*, *euryale* and *manto* form a compact cluster in the electrophoresis phenogram. Roos &

Arnscheid (1980a) have grouped *ligea*, *euryale* and *adyte* with *aethiops* (a in fig. 1) and *manto* and *eriphyle* with *melampus*, *sudetica* and *pharte* (b in fig. 1).

- *"pandrose* group" (9 in fig. 1). In the electrophoresis phenogram, *pandrose* is separated from *meolans* and *oeme*. According to the data of Roos & Arnscheid the three species are members of three different groups.
- *"pronoe* group" (8 in fig. 1). The data of Sonderegger are consistent with Roos & Arnscheid. Electrophoretically, however, *pronoe* and *montana* show very low similarities and both cluster with different species in the phenogram. *E. styx* was not available for electrophoretic analysis.
- *"epiphron* group" (2 in fig. 1). Sonderegger tentatively included *melampus* and *sudetica* in this group. This is not supported by the electrophoretic data. The grouping of Roos & Arnscheid is not consistent with either of the other two approaches.

The traditional approach of the systematist is to infer relationships from studies of phenotypic divergence of morphological characters. The electrophoretic approach may appear identical, except that the divergence is recorded at the molecular level. However, there are more important differences which concern the "characters", the enzyme proteins: it is a sample of homologous proteins which is compared over all taxa and which may be viewed as a sample of homologous genes. It is important in this context to point out that the genetic basis underlying the phenotypic change recorded is well understood. There is increasing evidence that a major part if not all of this variation is due to neutral or nearly neutral mutations. Therefore, the change observed proceeds in a stochastic fashion (Kimura, 1983) and is not governed by selective pressure. In fact, there is strong evidence that the levels of genetic relationship of the taxa compared (more commonly estimated in the literature as genetic distance D, which is related to the genetic identity I which we have used by $D = -\ln I$) are related to the time of divergence from a common gene pool (molecular clock hypothesis) (Kimura, 1983; for a review see e.g. Berlocher, 1984).

We are well aware of the fact that a more reliable estimate of genetic relationships requires the investigation of a large sample of homologous proteins. However, working on a variety of organisms over years, one experiences that trends may become evident from rather small samples

and we are very confident that the trends which are observed in this survey will hold as the number of loci scored increases.

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